REMARKS

Claims 1-37 are pending. Claim 38 has been withdrawn from consideration. Favorable consideration and allowance are respectfully requested for claims 1-37 in view of the following remarks.

Claim Objections

Claim 15 stands objected to for being misidentified as "currently amended" in the previous claim set. Applicant hereby submits a new claims set which properly identifies claim 15 as "previously presented."

Claim Rejections – 35 U.S.C. §103(a)

Claims 1-4, 14-31, 33 and 35-37 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107).

Gitan and Bransteitter

The Examiner is of the opinion that Gitan discloses a method for detecting the presence or level of alkylated cytosine in genomic DNA. The method disclosed in Gitan comprises converting the DNA to single stranded DNA, selectively modifying unmethylated cytosine to uracil using bisulfite and detecting the uracil in the DNA using a microarray. The Examiner concedes that Gitan does not disclose the use of an enzyme which selectively modifies unmethylated cytosine in single stranded DNA. However, the Examiner looks to Bransteitter to rectify this deficiency and alleges that in light of the combination of Bransteitter and Gitan the pending claims are obvious.

As a preliminary matter the Examiner states (at page 8, last paragraph of the Final Office Action) that both "Gitan et al and Bransteitter et al are interested in understanding the importance of methylation patterns in biological processes and therefore method steps are combinable".

The Applicant respectfully disagrees. Bransteitter identified activation-induced cytidine deaminase (AID) as a prerequisite for class-switch recombination and somatic hypermutation (SHM) in humans. The Applicant submits that this citation is not "interested in understanding the importance of methylation patterns". Rather, Bransteitter is clearly concerned only with class switch recombination and somatic hypermutation in B cells (see abstract) In particular, Bransteitter identifies a role for AID in SHM which is noted by the authors to be a process "which occurs only in the presence of RNA transcription" and which the present Applicant notes occurs only in B-cells. Thus it is erroneous to argue that Bransteitter is concerned with the importance of methylation patterns. The fact that AID deaminates unmethylated cytosine was identified only as part of the elucidation of the mechanism by which this enzyme works in SHM. Accordingly the Applicant submits that the Examiner's statement that both Gitan and Bransteitter are "interested in the importance of methylation pattern in biological processes" is incorrect and fails to establish any motivation to combine the method steps of Gitan with Bransteitter.

As Bransteitter is in no way concerned with detecting methylation patterns the Applicant submits that a skilled person would not have considered Bransteitter to be "reasonably pertinent" to the problem to be solved by the present application and therefore, would not have identified this reference or relied upon its contents. In particular, the Applicant submits that Bransteitter relates solely to the field of somatic hypermutation and it is not at all pertinent to the problem addressed by the present specification namely the development of methods to measure methylation and overcome the disadvantages associated with bisulfite modification. Moreover, as argued below the skilled person reading Bransteitter is in fact taught that the activity of AID is limited to its role in SHM.

Bransteitter teaches away from the present claims

Bransteitter states at page 4102, left hand column, third paragraph that "previous studies have failed to detect AID activity on either DNA or RNA" and goes on to identify that AID in fact acts selectively at 9-nucleotide "bubbles". In that regard Bransteitter also states at page 4160, right hand column, first full paragraph that the 9-nucleotide bubble was used because "SHM occurs only in the presence of RNA transcription". Additionally Bransteitter clearly sets out, for

example at Figure 1 and page 4104 left hand column, first and second full paragraphs, that AID only functions when pretreated with RNase. This is wholly consistent with, and provides to the reader further evidence that the role of AID is in SHM. As is recognized by Bransteitter "SHM occurs only in the presence of RNA transcription" and it is entirely logical therefore for AID to require RNA to be degraded by RNase before it can access and act on the partially single-stranded DNA bubble generated during active transcription.

The Applicant therefore submits it is clear that Bransteitter teaches a role for AID that is limited to somatic hypermutation (SHM). Further, SHM is known to occur only in B-cells and specifically only at the regions of genes encoding the variable regions of antibodies. Bransteitter, at page 4104, left hand column, first paragraph and the first paragraph of the right hand column state that AID shows greater activity in "5'-AGCT SHM hot-spot sequences compared with 5'-ATCT and 5'-GACA non-hot-spot sequences" and note that the available data suggests that "AID activity depends on sequence context".

Bransteitter teaches away from the use of single stranded DNA as the activity of the AID enzyme is far greater on partially single stranded DNA bubbles (see for example Table 1 of Bransteitter at page 4106, right hand column) where it is indicated that AID has the greatest activity on a 9 nucleotide bubble "corresponding to a typical transcription bubble size" (Bransteitter at page 4106, right hand column, first full paragraph). The Applicant submits this is consistent with the fact noted by Bransteitter that somatic cell hypermutation occurs only during RNA transcription where a "transcription bubble" exists and is also consistent with the finding by Bransteitter that RNase activity is essential for AID activity. The Applicant submits that because Bransteitter teaches that AID requires active transcription a skilled person would not expect that AID will work in the absence of RNA transcription.

The Applicant submits that the skilled person would, in view of Bransteitter, not consider the use of AID as a means to differentially modify alkylated cytosine and cytosine present in genomic or mitochondrial DNA as Bransteitter teaches that AID is selectively active on 9 nucleotide bubbles. In addition Bransteitter use short synthetic DNA of defined sequence in their

experiments and not fragments of genomic DNA of as required by the claims of the present application which may have a sequence that is not apparent when performing the claimed method. Therefore Bransteitter provide no indication that the AID enzyme is functional on fragments of genomic DNA. Indeed, the Applicant submits that in view of Bransteitter's teachings that AID has a sequence preference for SHM hot-spots, a preference for 9-nucleotide "bubbles" and requires RNase pretreatment to function (thus suggesting the need for active transcription) strongly indicates that AID would not be functional on fragments of genomic DNA, nor outside the normal physiological intracellular environment in which transcription is actively proceeding. Therefore a skilled person would not consider that AID would function with the isolated DNA (i.e. the DNA obtained from an individual) required by the present claims. Accordingly a skilled person would not be motivated to extrapolate the teachings of Bransteitter that AID functions with greatest efficiency on short synthetic 9-nucleotide bubbles, to the use of AID on fragments of genomic DNA with a reasonable expectation of success.

In addition, a skilled person would not seek to use the AID enzyme to investigate the methylation status of genomic DNA as AID enzymes were, at the time the present invention was made, known to be sequence specific. For example, the present specification as filed on page 8, line 1 to page 8, line 25 discuses the sequence specificity of AID enzymes. Accordingly a person skilled in the art looking to detect alkylated cytosine in DNA, rather than at specific sites within DNA, would not consider using AID as the sequence-specificity of that enzyme would be a disadvantage. In view of this the Applicant submits the skilled person before this present invention would not have used AID to modify unmethylated cytosine because the sequence specificity of AID would be understood to lead to inconsistent results. Therefore for at least this reason AID would not have been considered as a modification of the bisulfite modification step of Gitan as per the Examiner's position.

The Examiner considers that Bransteitter teaches that both AID and RNaseA in combination are employed to differentially modify 5-methyl cytosine and cytosine. The Applicant disagrees and notes that RNaseA is required to remove inhibiting RNA and plays no role in the differential modification of cytosine. This is consistent with a role for AID in somatic

hypermutation as identified by Bransteitter and which occurs only in transcriptionally active sites.

Thus, in view of the foregoing the Applicant submits a skilled person in view of Bransteitter would not consider that AID would to be useful in the claimed method of detecting the presence or level of alkylated cytosine in a sample of genomic or mitochondrial double-stranded DNA.

Modification of deamination step not obvious

The Examiner states that it would have "been obvious to modify the bisulfite deamination step of Gitan et al with the expected benefit of using the AID enzyme". The Applicant respectfully disagrees at least because the bisulfite modification step of Gitan takes place in the presence of sodium hydroxide at 50°C. Specifically, the protocol followed by Gitan (the Intergen kit, as noted by the Examiner) requires the use of three microlitres of concentrated sodium hydroxide (3 molar) in a total reaction volume of 107 microlitres. The Applicant submits this equates to 196 mM sodium hydroxide with a calculated pH of 13.29. At this pH and sodium hydroxide concentration a skilled person would understand that the AID enzyme would be destroyed and therefore incapable of any function let alone the deamination of cytosine. Therefore the skilled person would not consider it obvious to modify the bisulfite deamination step of Gitan. Clearly there would be no expected benefit of using the AID enzyme in an environment that would obviously destroy the enzyme. Further, the Applicant submits the presence of sodium hydroxide and heat in the bisulfite modification step of Gitan is required as the removal of the sodium hydroxide and cooling of the sample would result in re-annealing and/or precipitation of the DNA such that single stranded DNA would no longer be present, or a significantly reduced amount such that no meaningful analysis of the methylation state of the DNA could be performed. Accordingly, the Applicant submits a skilled person would not consider it "obvious to modify the deamination step of Gitan et al".

Present Application solves different problem

The Examiner has stated that the problem to be solved by the present invention is one of decreasing the time to modify methylated cytosine. Thus a skilled person would "like to use an enzyme for detecting methylation status in a target genome, because it requires less time and does not require additional steps of purifying target".

With respect, the Applicant considers that the Examiner has misconstrued the problem to be solved by placing an undue emphasis on the reduction of time and lack of purified target aspects of the problem. The Applicant notes that on page 4, line 2 to page 5, line 8 of the present specification as filed a further problem to be solved by the present invention is noted as the avoidance of artifacts which can arise in bisulfite based methods. Thus, the Applicant submits that the present invention also aims to solve the problem of artifacts of bisulfite modification. The Applicant respectfully submits that in light of this the combination of Gitan and Bransteitter is inappropriate as neither citation teaches or suggests the use of AID may be appropriate or useful to avoid the artifacts associated with bisulfite treatment.

Further the Applicant submits that Bransteitter states that "previous studies have failed to detect AID activity on either DNA or RNA, the biologically relevant substrate for AID remains an important open question". Thus, Bransteitter, by teaching at page 4106 Table 1 that AID acts selectively on 9-nucleotide bubbles, by teaching at page 4104 that AID displays a preference for SHM hot-spot sequences, and by teaching that RNase pretreatment is required for AID activity is clearly a document that simply elucidates the biologically relevant substrate for AID within the context of SHM. That is, Bransteitter is not relevant and would not be considered relevant by those skilled in the art to the problems addressed by the present invention.

Combination made in hindsight

The Applicant respectfully submits that the Examiner is using hindsight to make the combination of Gitan and Bransteitter. The Applicant submits that in full view of the claims the Examiner has merely searched for references which broadly disclose some of the steps of the claimed method (Gitan) and subsequently searched for another reference which allegedly provides the missing feature (Bransteitter). The Applicant argues that such hindsight analysis of

the invention is impermissible and therefore the combination of Gitan and Bransteitter is also impermissible.

The Examiner also rejects Claims 5-13 are rejected under 35 U.S.C. § 103 as allegedly unpatentable over Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107) further in view of Kuhn *et al.* (*J. Am. Chem. Soc.*, 2002, 124, 1097-1103).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Kuhn *et al.* provide methods for separating double stranded DNA into two single strands. Kuhn *et al.* do not, however, provide any teaching or information regarding differentially modifying alkylated cytosine and cytosine present in single stranded DNA. Accordingly, it is respectfully submitted that the addition of Kuhn *et al.* do not cure the deficiencies of the combination of Gitan *et al.* and Bransteitter *et al.* Therefore, it is respectfully submitted that the claims are inventive over the combination of Gitan *et al.* in view of Bransteitter *et al.* further in view of Kuhn *et al.*

The Examiner has also rejected Claim 32 for allegedly being obvious in light of Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107) further in view of Opdecamp *et al.* (*Nucleic Acids Research*, 1992, 20, 171-178).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Opdecamp *et al.* teach the identification of methylated DNA using methylation-sensitive restriction enzymes. In the claimed method the enzyme is acting on single stranded DNA. It is well understood that restriction enzymes only recognize and cleave double stranded DNA. Accordingly, none of the enzymes referred to in Opdecamp *et al.* differentially modify cytosine and alkylated cytosine in single stranded DNA. Accordingly, it is respectfully submitted that the present claims are inventive in view of the combination of Gitan *et al.* in view of Bransteitter *et al.* further in view of Opdecamp *et al.*

The Examiner has also rejected Claim 34 under 35 U.S.C. § 103 as allegedly being unpatentable over Gitan *et al.* (Genome Research, 2001, 12, 158-164) in view of Bransteitter *et*

al. (PNAS, 2003, 100, 4102-4107) further in view of Paulson *et al.* (J. Virol., 1999, 73, 9959-9968).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Paulson *et al.* use bisulfite modified DNA which is then amplified using PCR to detect sites of methylation in the viral genome. Accordingly, the methodology of Paulson *et al.* is similar to that of Gitan *et al.* and which fails for the same reasons. As with Gitan *et al.* there is no disclosure in Paulson *et al.* for the use of an enzyme which differentially modifies cytosine and alkylated cytosine. Further, the use of an enzyme in the methods described by Paulson *et al.* would also render their methods as unsatisfactory for the intended purpose i.e. the method would fail to work.

Therefore, in view of the foregoing remarks, withdrawal of the rejections raised under 35 U.S.C. § 103 are respectfully requested.

CONCLUSION

In view of the foregoing, the application is respectfully submitted to be in condition for allowance, and prompt favorable action thereon is earnestly solicited.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

The fee in the amount of $1,920.00 is being paid simultaneously herewith via EFS representing payment of a three-month extension of time pursuant to 37 C.F.R. §1.17(a)(3) ($1,110.00) and payment of a Request for Continued Examination pursuant to 37 C.F.R. §1.17(e) ($810.00). Please charge any deficiency in fees or credit any overpayments to Deposit Account No. 50-3211 (Docket No. 22238.0004).

Respectfully submitted,

Date: February 28, 2011

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